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## METHOD FOR TREATING ONYCHOMYCOSIS

This application is a divisional under 35 U.S.C. § 120 of U.S. Non-Provisional application Ser. No. 10/031,929 filed 25 Jan. 2002, now abandoned, which was a National Stage filing under 35 U.S.C. § 371 of PCT/JP00/04617 filed 11 Jul. 2000, which claimed priority to Japanese patent application Ser. No. 11/214,369 filed 28 Jul. 1999.

### TECHNICAL FIELD

The present invention relates to a method for detecting pathogenic microorganism, method for evaluating an effect of an antimicrobial agent on pathogenic microorganism and a method for detecting an antimicrobial agent. The present invention also relates to an antimicrobial agent and a therapeutic agent for onychomycosis, which are obtained according to the above-mentioned method for evaluating the drug effect.

### BACKGROUND ART

A method for evaluating a drug effect with an animal model is needed in order to explore a novel antimicrobial agent (also hereinafter referred to "drug"). Further, a method enabling a drug effect to be evaluated with accuracy is needed because of grate importance in view of predicting a clinical therapeutic efficiency thereof.

Historically, an experimental dermatophytosis model that back, planta and interdigital of a guinea pig have been infected with *Trichophyton mentagrophytes* has been used in order to evaluate an effect of an antifungal agent on dermatophytosis. Such animal models have been already employed to develop some antifungal agent. The evaluation of the effect of such antifungal agent carried out by applying the antifungal agent to the infected animal, by excising the skin after the certain period of time to cut into plural small pieces, by cultivating the skin pieces on the medium, and by counting the number of pieces wherein no growth of fungus is seen or the number of animals or feet wherein no growth of fungus is seen in all skin pieces, as an indicator (Antimicrobial Agents and Chemotherapy, 36: 2523-2525, 1992, 39: 2353-2355, 1995). Hereinafter, the conventional method for evaluating the drug effect is referred to as "the conventional method".

Although the drug having a potent activity against *Trichophyton* in vitro such as lanconazole or amorolfine has been marketed in these days, an improvement of cure rate in a clinical use is hardly seen. As a main reason thereof, a relapse that since fungus in the skin is not completely killed after a treatment, the fungus grow again is pointed.

In also animal experiments, when an effect of lanconazole on guinea pig models of tinea pedis was evaluated using the conventional method, though "fungus-negative" was observed in all feet out of 20 feet 2 days after the last treatment, a relapse was observed in 11 out of 20 feet 30 days after the last treatment, and no correlation was seen between the effect 2 days after the last treatment and the effect 30 days after the last treatment (36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La., 1996, Abstr. F80).

As a reason thereof, there were followings. Since lanconazole have very potent antitrichophyton activity in vitro, lanconazole persisted in the skin 2 days after the last treatment in the concentration wherein the sterilization effect was shown. Therefore, when the skin is excised and culti-

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vated on the medium to detect fungus, the lanconazole remaining in the skin is diffused in the medium, and therefore, no fungus was detected due to prevention of the growth regardless of the presence of viable fungus in the excised skin. On the other hand, since the concentration of the drug remained in the skin is reduced 30 days after the last treatment, fungus in the skin can grow again and can be detected by culture study.

According to this hypothesis, it is ascertained that the drug remain in the skin through the inhibition of the growth of fungus around the skin blocks completely, when the lanconazole-treated skin blocks were located and cultivated on the medium which contains dermatophytes.

Therefore, it became to clear that the conventional method has the problem that the drug effect can not be accurately evaluated, because the apparent therapeutic effect need to be evaluated after removing the drug remaining in the skin.

Meanwhile, a kind of mycosis, dermatophytosis, is the superficial dermatosis which is caused by dermatophyte parasitizing the keratin such as skin (stratum corneum), the nail and the hair. In particular, tinea unguium formed in the nail is known as the intractable disease among dermatomycoses based on dermatophytoses, and is accompanied by symptom such as opacity, tylosis, destruction and deformation of nail plate. Now the oral preparation (such as griseofulvin or terbinafine) is used for the treatment of such tinea unguium. However, there are many cases where the patient stops taking the drug or that takes the drug irregularly, since they have to take the drug for a long period, for example at least a half a year in order to completely cure tinea unguium. It is thought that this is a main cause of difficulty of curing tinea unguium completely. Furthermore, by taking the drug for a long period, griseofulvin has the problem of side effects on internal organ (gastrointestinal disorder, hepatotoxicity) and hepatotoxicity is reported as the side effect in terbinafine. Therefore, in order to improve the compliance of the patient it is desired to develop a topical preparation which cure tinea unguium for a short period and has less the systemic side effect than the oral preparation.

However, in case of the simple application on nail plate with the current antifungal agent for topical use, the antifungal effect on fungus in the nail was not seen, because the drug could not sufficiently permeate the thick keratin in nail plate (Markus Niewerth and Hans C. Kortling, Management of Onychomycoses, Drugs, 58: 283-296, 1999).

In addition, the therapeutic effect of a topical preparation of antifungal agent on the experiment model of trichophytosis can not be evaluated using the conventional method as mentioned above. This may be a reason why the drug effect on the guinea pig model of tinea unguium has been hardly reported.

### DISCLOSURE OF INVENTION

The present invention has been accomplished based on findings that it is desirable that an effect of antimicrobial agent such as particularly antifungal agent is evaluated after removing a drug remaining in the infected site after treatment of an animal or a biosample such as skin with the pathogenic microorganism. An object of the present invention is to provide a novel method for evaluating the effect of the antimicrobial agent and the antimicrobial agent obtained according to the method for evaluating the drug effect. In detail, the present invention provides the method for detecting the viable pathogenic microorganism in the above-mentioned infected site of the animal or the biosample with the pathogenic microorganism after removing the antimi-